Molecular Biology and Biotechnology

A Comprehensive Desk Reference

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ture, being more akin to valleys and grooves than to deep crevices (Figure 5A). Amino acid composition of binding sites is biased toward polar side chains with high dipole moment (Asn, Gln) and aromatic rings—that is, groups well capable of neutralizing the predominantly polar and charged antigenic epitopes (see Section 6.1).

Biophysical importance of an overall concave shape of binding sites relates to the following facts: (1) diffusion away from cavities is significantly slower than from flat surfaces, or through the solvent; (2) (attractive) electrostatic fields are enhanced or focused in cavities, even though solvent quenches the field at other flat or convex parts of protein surface (Figure 5B,D), and (3) hydrophobicity of a surface is a function of its curvature relative to the size and curvature of a water molecule; thus, concave surfaces are more hydrophobic than flat or convex surfaces, providing more "hydrophobic binding energy" (Figure 5C).

6 MOLECULAR PROPERTIES OF ANTIGENS: ANTIGENICITY

6.1 Humoral (Antibody) Antigenicity

Classical immunochemical experiments have shown that virtually any chemical structure, attached to a protein molecule, can elicit antigenic response. The vast scientific literature focused on antigenic properties of proteins contains several alternative, and often contradictory, theories of antigenicity.

The term *antigenicity* refers to the ability of a protein surface region to be potentially antigenic, while *immunogenicity* refers to the ability of any antigenic site to elicit such a response under particular circumstances (immunization protocol, genetic constellation of the organism, etc.).

Antiprotein antibodies sometimes specifically recognize short peptides (tetra-, penta-, hexapeptides); such antibodies can be elicited by synthetic peptide antigens. The majority of antigenic sites in proteins, however, seem to consist of amino acids that are not contiguous in the amino acid sequence (composite or discontinuous epitopes).

A concept of a "functional (energetic)" epitope, originally formulated in computational analysis of X-ray crystallographic structures, has recently found support in extensive alanine-scanning mutagenesis experiments of antigen–antibody complexes. Thus, only a small part of the total antibody and antigen contact surface (some 30–40% thereof, or three to four amino acid residues) appears to contribute actively to binding, often via hydrogen bonds and buried salt links. This smaller contact area (a functional, or energetic, epitope) belongs to the most protruding parts of the antigenic surface, where side chains such as Arg, Glu, Gln, Lys, and Pro are particularly abundant. For the complex formation to be possible, however, there must be complementarity between additional surface area of the antibody and the antigen. Hence we arrive at different operational definitions of antigenicity, depending on whether we emphasize energetics of complex formation, complementarity of antigen–antibody surfaces, or other phenomena. The conjecture that antigenicity correlates with surface protrusion provides a natural link between the two extreme antigenicity theories ("distinct antigenic epitopes exist" vs. "the whole surface is antigenic") by introducing the concept of antigenic probability that varies along the surface.

6.2 Cellular (T-Cell Receptor) Antigenicity

Structural problems related to cellular antigenicity are similar to those of humoral antigenicity: we wish to identify those structural traits of a peptide that make it capable of eliciting a cellular immune response. Molecular mechanisms of T-cell receptor recognition, however, involve antigen presentation by the MHC molecules, and phenomenology of cellular antigenicity is therefore distinctly different from that of the humoral antigenicity.

T-Cell antigens are peptides derived from proteins that became sequestered by presenting cells, after which they were intracellularly cleaved, and then incorporated into the MHC protein structure in the process of its folding. Peptides presented in the MHC peptide-binding groove and specifically recognized by T-cell receptors are often derived from polypeptide segments buried inside foreign protein antigens. The majority of the MHC allelic amino acid replacements modify the shape of the peptide-presenting groove, thereby determining broad classes of peptide sequences that are compatible or incompatible with that allele. Thus, the main determinants of cellular antigenicity are a suitable length (an optimal range for fitting comfortably into the presenting groove appears to be 8–11 residues) and an amino acid sequence compatible with tight binding to an MHC allele.

7 PROTEIN ENGINEERING IN IMMUNOLOGY

The modular three-dimensional design of immunoglobulins, T-cell receptors, and MHC antigens lends itself ideally to protein engineering schemes that shuffle, transpose, and reconnect various independent folding units (domains) of various proteins (Figure 6). Protein engineering of immunoglobulins and T-cell receptors has blossomed since middle 1980s, taking advantage of computer-aided structural design based on available X-ray crystallographic coordinates of many antibodies, rapid development of gene cloning technologies, which allowed subcloning of eukaryotic genes into bacterial plasmids, and progress in controlled bacterial expression of proteins from plasmid-inserted genes. Expression vectors such as yeast, baculovirus, and mouse hybridoma cells have been used; large yields of protein chimeras from inexpensive bacterial (*E. coli, Streptomyces* sp.) cultures are becoming common. Proteins are obtained either as secreted, refolded, soluble species or as insoluble, denatured intracellular aggregates—"inclusion bodies" that are easy to isolate but must be solubilized and renatured before a functional protein is obtained.

7.1 Stable Fragments, Domain Swaps, Immunotoxins

Some of the first man-made chimeras were mouse antigen-binding domains (V_L and V_H) implanted on human constant region domains, V_H domains spliced onto C_L domains giving rise to functional, antigen-specific, V_H-C_L/V_L-C_L light-chain-like dimers, and recombinant mouse antibodies with novel effector functions engineered via H-chain constant domain swaps, deletions (to produce $[Fab]'_2$-like, disulfide-bonded bivalent molecules), and replacements with foreign protein domains (e.g., enzymatically active Fab-staphylococcal nuclease or Fab-*myc* oncogene constructs). Production of large quantities of stable Fv fragments in bacteria has been aided by the design and successful preparation of a single-chain Fv whereby the C-terminus of a V_H (alternatively, V_L) domain is connected to the N-terminus of the V_L (V_H) domain by a short

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fibrillar protein) do not tend to react well with denatured preparations and this is consistent with the view that the majority recognize topographical (surface) structures (i.e. epitopes) which depend upon the conformation of the native molecule. For this reason, antibodies to native proteins do not usually react as strongly with peptides having the same primary sequence (figure 4.2). When individual epitopes are mapped using homogeneous monoclonal antibodies (cf. p. 107), they are frequently seen to involve amino acid residues far apart in the primary sequence, but brought together by the folding of the peptide chains in the native protein (figures 4.3 and 4.6). It seems reasonable to talk of *discontinuous* or assembled rather than *continuous* or sequential epitopes in these cases.

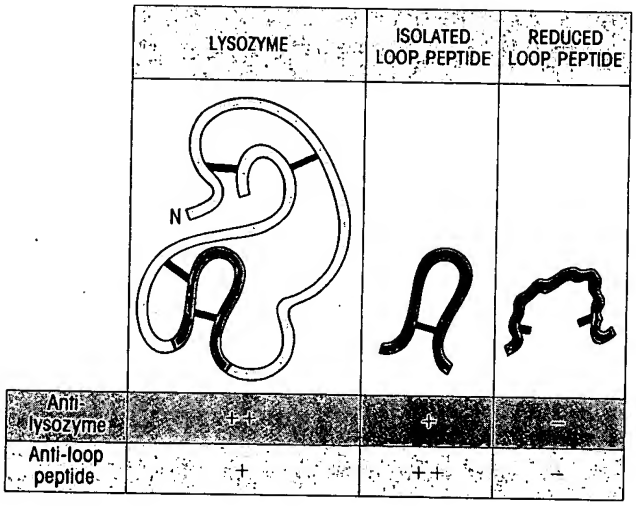

Figure 4.2. *Specificity and three-dimensional configuration in a globular protein, lysozyme. Antibodies to the whole molecule and to the isolated loop peptide do not react with the peptide after reduction of its disulphide bond, showing that the linear reduced peptide has lost the antigenic configuration it had when held as a loop even though the amino acid sequence was unchanged (from Maron E., Shiowa C., Arnon R. & Sela M. (1971)* **Biochemistry** *10, 763).*

If one were to take each individual antibody within an antiserum raised to a protein antigen and plot the approximate centre of the corresponding epitope on the antigen surface, one would almost certainly finish up with a 'contour map' of epitope density indicating regions on the antigen surface of *dominant epitope clusters* (figure 4.4a). Each of these clusters is as near as I can get to defining an antigen *determinant*. It is important to be aware that each antigen usually bears several determinants on its surface, which may well be structurally distinct from each other; thus a monoclonal antibody reacting with one determinant will usually not react with any other determinants on the same antigen unless the molecule has axes of symmetry (figure 4.4b).

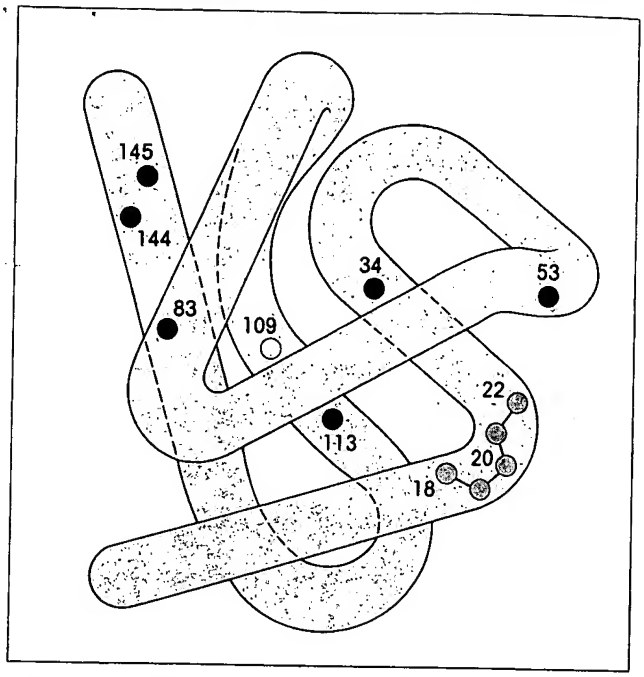

Figure 4.3. *Epitope residues on the folded peptide chain of sperm whale myoglobulin. Amino acid residues 34, 53, and 113 (●) contribute to the epitope recognized by one homogeneous monoclonal antibody, 83, 144 and 145 (●) to another. These are clearly* **discontinuous** *epitopes. Amino acids 18–22 (○) are postulated to form part of a continuous epitope based on reactions with the isolated peptides. Much of the myoglobin chain is in the α-helical form. Residue 109 (○) is critical for T-cell recognition and so far no antibodies reacting with this site have been demonstrated. (Based on Benjamin* **et al.** *(1986)* **Ann. Rev. Immunol.** *2, 67.)*

Antigens and antibodies interact by spatial complementarity not by covalent bonding

Once a method had been found for raising antibodies to small chemically defined haptens (figure 4.1), it then became possible to relate variations in the chemical structure of a hapten to its ability to bind to a given antibody. In one experiment, antibodies raised to *m*-aminobenzene sulphonate were tested for their ability to combine with *ortho*, *meta* and *para* isomers of the hapten and related molecules in which the sulphonate group was substituted by arsonate or carboxylate (table 4.1). The hapten with the sulphonate group in the *ortho* position combines somewhat less well with the antibody